Antioxidative Phenolic Compounds from Sage (*Salvia officinalis*)

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Ten phenolic compounds were isolated from a butanol fraction of sage extracts. Their structures were determined by spectral methods (NMR, MS, IR). Among them, a novel compound, 4-hydroxyacetophenone-4-*O*-*β*-D-apiofuranosyl-(1→6)-*O*-*β*-D-glucopyranoside, was identified. Two test systems, DPPH free radical scavenging activity and radical cation $ABTS^{\bullet+}$ scavenging activity, were used to evaluate their antioxidant activity. The most active compounds were found to be rosmarinic acid and luteolin-7-*O*-*β*-glucopyranoside.

Keywords: *Sage; Salvia officinalis; phenolic compounds; antioxidant activity*

INTRODUCTION

The addition of antioxidants to food is an effective way to prevent the development of various off-flavors and undesirable compounds that result from lipid oxidation. The possible toxicity as well as general consumer rejection led to decreasing use of synthetic antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) (Namiki, 1990). Antioxidants with natural origins, therefore, have drawn more and more attention. Among the plants reported to have antioxidative activities, rosemary and sage are widely known. The main antioxidant activity of sage was reported to be attributed mainly to carnosic acid, carnosol, and rosmarinic acid (Cuvelier et al., 1996). However, the chemical components of sage are very complex. Many components such as diterpenes, triterpenes, and flavonoids have been isolated from sage (Brieskorn and Buchberger, 1973; Brieskorn and Kapadia, 1979, 1980; Karl et al., 1982; Djarmati et al., 1992; Cuvelier, 1994, 1996; Tada, 1997). Most of the compounds are phenolic compounds and may have a significant contribution to the total antioxidant activity of sage. Since there is no report on the highly polar components of sage, we examined the chemical components of the butanol-soluble fraction of sage. Here we report the structures of 10 phenolic compounds and their antioxidative activity in two test models.

MATERIALS AND METHODS

General Procedures. ^{1}H NMR and ^{13}C NMR spectra were obtained on a VXR-200 and 300 instrument. Desorption chemical ionization mass spectra were measured on a JEOL SX-102 mass spectrometer using ammonia as a reactant gas. FAB mass spectra were recorded on a Finnigan MAT-90 instrument. FT-IR spectra were obtained on a Perkin-Elmer 1600 apparatus. The spectra were measured in KBr pellets. Optical rotations were recorded on a JASCO DIP-181 polarimeter. Thin-layer chromatograghy was performed on Sigma-Aldrich silica gel TLC plates (250 μm thickness, 2−25 μm particle size), with compounds visualized by spraying with 5% (v/v) H_2SO_4 in an ethanol solution. Silica gel (130−270 mesh), Sephadex LH-20 (Sigma Chemical Co., St. Louis, MO), and Lichroprep RP-18 columns were used for column chromatography. Radical 2,2-diphenyl picryhydrazyl (DPPH) and silica gel (130−270 mesh) were purchased from Aldrich Chemical Co. (Milwaukee, WI). A total antioxidant status kit was purchased from Randox Laboratories Ltd. (San Francisco, CA). All solvents used for chromatographic isolation were analytical grade and were purchased from Fisher Scientific (Springfield, NJ).

Plant Material. The leaves of sage were a gift from Kalsec Inc. (Kalamazoo, MI).

Extraction and Isolation Procedures. The dried leaves of sage (30 kg) were extracted with 95% ethanol (50 L) at room temperature for 2 weeks. The extract was concentrated to dryness under reduced pressure; the residue was dissolved in water (2.5 L) and partitioned with hexanes (3 × 3 L). Then the water layer was extracted successively with ethyl acetate (3 × 3 L) and 1-butanol (3 × 3 L). The water-saturated 1-butanol extract was evaporated in vacuo to give 320 g of residue. The residue was subjected to column chromatography (CC) on silica gel and eluted with chloroform−methanol as eluent with increasing methanol content (20:1, 15:1, 10:1, 9:1, 7:1, 5:1, 4:1, 2:1, 1:1, each 5 L), and 1-L fractions were collected and concentrated to dryness under reduced pressure. A total of 45 fractions (fractions 1−45) were collected.

Fractions 10−12 (20 g) were combined and first subjected to a Sephadex LH-20 column (eluted with methanol) and then rechromatographed on a Lichroprep RP-18 column using methanol−water (3:7) to get four fractions (I−IV). Fraction I was then subjected to a Lichroprep RP-18 column eluted with methanol and water (1:3 1000 mL then 3:7) to get two fractions IA and IB. Fraction IB was subjected to a silica gel column eluted with ethyl acetate−methanol−water (12:1:1) to get 600 mg of compound **1**.

Fraction 18 (10 g) was subjected to an RP-18 column using methanol−water (1:3) as eluent to get four fractions I−IV. Fraction I was first subjected to a Sephadex LH-20 column (eluted with methanol) and then purified with a silica gel column eluted with chloroform−methanol−water (6:1:0.1) to get 45 mg of compound **2** and 140 mg of compound **3**. Fraction

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10.1021/jf980614b CCC: $15.00
Published on Web 11/17/1998

Table 1. ^{13}C NMR Data of Compounds 2, 8, and 9

position	compd **2**[a]	compd **8**[a]	compd **9**[a]
1	26.9 (q)	26.8 (q)	139.2 (s)
2	200.2 (s)	199.8 (s)	129.6 (d)
3	133.0 (s)	132.9 (s)	129.6 (d)
4	132.1 (d)	132.0 (d)	129.0(d)
5	117.6 (d)	117.6 (d)	129.6 (d)
6	163.4(s)	163.3 (s)	129.6 (d)
7	117.6 (d)	117.6 (d)	72.1(t)
8	132.1(d)	132.0 (d)	
1′	101.8 (d)	101.9 (d)	103.5(d)
2′	75.1 (d)	75.1 (d)	75.3(d)
3′	78.2 (d)	78.3 (d)	78.3 (d)
4′	71.3 (d)	71.8 (d)	72.1 (d)
5′	78.7 (d)	77.5 (d)	77.3 (d)
6′	62.8 (t)	69.1 (t)	69.0 (t)
1″		111.3 (d)	111.3 (d)
2″		78.2 (d)	78.3 (d)
3″		80.8 (s)	80.9 (s)
4″		75.3(t)	75.4 (t)
5″		65.7 (t)	65.9 (t)

[a] Measured in CD_3OD.

II was rechromatographed on silica gel CC and eluted with chloroform−methanol−water (7:1:0.1) to get 18 mg of compound **4** and 18 mg of compound **6**. A total of 400 mg of compound **5** was crystallized from fraction IV.

Fractions 21 and 22 (15 g) were combined and subjected to a Lichroprep RP-18 column eluted with methanol−water (1:3 1000 mL, 3:2 600 mL) and methanol (500 mL) to get three fractions. Fraction I was subjected to a Sephadex LH-20 column (eluted with methanol) to get two fractions: fraction IA and 100 mg of fraction IB (compound **7**). Fraction IA was rechromatographed on silica gel CC (eluted with ethyl acetate−methanol−water, 10:1:1) to get six fractions; the fourth fraction was subjected to silica gel CC eluted with methylene chloride−methanol−water (5:1:0.1) to get 40 mg of compound **8**. The third fraction was first subjected to a silica gel column (eluted with chloroform−methanol−water, 5:1:0.1) and then purified on a Sephadex LH-20 column to get 170 mg of compound **9** and 35 mg of compound **10**.

Spectrometric Identification of Isolated Compounds. *Rosmarinic acid (**1**).* Amorphous powder; DCI−MS *m/z*: 378 $[M + NH_4]^+$; ^{1}H NMR (in CD_3COCD_3, 300 MHz): δ 3.03 (2H, m, H-7′), 5.20 (1H, m, H-8′), 6.29 (1H, d, J = 16.0 Hz, H-8), 6.61−6.87 (4H, m, H-5, H-2′, H-5′, H-6′), 7.03 (1H, dd, J = 8.0, 2.0 Hz, H-6), 7.16 (1H, d, J = 2.0 Hz, H-2), 7.55 (1H, d, J = 16.0 Hz; H-7). ^{13}C NMR (in CD_3COCD_3, 75 MHz): δ 171.2 (s, C-9′), 166.8 (s, C-9), 149.0 (s, C-4), 146.5 (s, C-3), 146.3 (d, C-7), 145.7 (s, C-3′), 144.7 (s, C-4′), 129.1 (s, C-1′), 127.3 (s, C-1), 122.7 (d, C-6), 121.6 (d, C-6′), 117.3 (d, C-2′), 116.3 (d, C-5), 115.5 (d, C-5′), 115.2 (d, C-2), 114.8 (d, C-8), 73.7 (d, C-8′), 37.4 (t, C-7′) are identical with the literature (Tezuka et al., 1998; Kikuzaki et al., 1989).

*4-O-β-D-Glucopyranosylacetophenone (picein, **2**).* Amorphous powder; DCI−MS *m/z*: 316 $[M + NH_4]^+$; ^{1}H NMR (300 MHz, CD_3OD): δ 7.98 (2H, d, J = 8.0 Hz, H-4 and H-8), 7.16 (2H, d, J = 8.0 Hz, H-5 and H-7), 5.04 (1H, d, J = 7.2 Hz, H-1′), 3.90 (1H, m, H-6′), 3.71 (1H, dd, J = 12.0; 5.6 Hz, H-6′), 3.31−3.52 (4H, m, H-2′; H-3′; H-4′ and H-5′), 2.56 (3H, s, H-1); ^{13}C NMR (see Table 1) are identical with the literature (Ushiyama and Furuya, 1989).

*6-O-(E)-Feruloyl-(α and β)-glucopyranoside (**3**).* Amorphous powder; DCI−MS *m/z*: 374 $[M + NH_4]^+$; ^{1}H NMR (DMSO-d_6, 200 MHz): 7.57 (d, J = 16.0 Hz, H-7), 7.36 (br s, H-2), 7.15 (br d, J = 8.1 Hz, H-6), 6.81 (d, J = 8.1 Hz, H-5β), 6.70 (d, J = 6.4 Hz, H-5α), 6.51 (1H, d, J = 16.0 Hz, H-8β), 6.50 (d, J = 16.0 Hz, H-8α), 4.20−5.20 (m), 3.84 (s, OCH_3), 3.00−3,80 (m). ^{13}C NMR (DMSO, 75 MHz): δ 166.8 (s, C-9), 149.5 (s, C-3), 148.0 (s, C-4), 145.3 (d, C-7), 125.5 (s, C-1), 123.4 (d, C-6), 115.5 (d, C-5), 114.3 (d, C-8), 111.1 (d, C-2), [97.0 (d,), 76.4 (d), 74.7 (d), 73.6 (d), 70.2 (d), 63.9 (t)] for β-Glc, [92.3 (d), 72.9 (d), 72.2 (d), 70.6 (d), 69.3 (d), 63.9 (t)] for α-Glc and 55.7 (q, OCH_3) are identical with the literature (Bokern et al., 1991).

*(+)-1-Hydroxypinoresinol-1-β-D-glucoside (**4**).* Amorphous powder; $[\alpha]_D^{22}$ −18.2 °C (c 0.11, CD_3OD); DCI−MS *m/z*: 554 $[M + NH_4]^+$; ^{1}H NMR (300 MHz, CD_3OD): δ 6.60−7.11 (6H, m, arom. H), 3.89 (3H, s, OCH_3), 3.85 (3H, s, OCH_3), 3.60−4.90 (m), 2.90−3.55 (m). ^{13}C NMR (CD_3OD, 75 MHz): 149.4 (s, C-3″), 148.5 (s, C-3′), 147.5 (s, C-4″), 147.4 (s, C-4′), 133.2 (s, C-1″), 128.8 (s, C-1′), 122.4 (d, C-6′), 120.0 (d, C-6″), 116.4 (d, C-5″), 115.4 (d, C-5′), 114.2 (d, C-2′), 111.0 (d, C-2″), 100.1 (d, C-Glc1), 99.2 (s, C-1), 89.9 (d, C-2), 87.0 (d, C-6), 78.4 (d, C-Glc3), 78.0 (d, C-Glc5), 74.9 (d, C-Glc2), 73.5 (t, C-8), 72.2 (t, C-4), 71.3 (d, C-Glc4), 62.6 (t, C-Glc6), 60.2 (d, C-5), and 56.7 (q, 2 CH_3) are identical with the literature (Tsukamoto et al., 1985; Wang et al., 1993).

*Homoplantaginin (**5**).* Yellow powder; DCI−MS *m/z*: 463 $[M + 1]^+$; ^{1}H NMR (DMSO-d_6, 300 MHz): δ 7.94 (2H, d, J = 8.8 Hz, H-2′and H-6′), 7.00 (1H, s, H-8), 6.95 (2H, d, J = 8.8 Hz, H-3′and H-5′), 6.94 (1H, s, H-3), 5.10 (1H, d, J = 7.4 Hz, H-Glc1), 3.10−3.80 (m), 3.75 (3H, s). ^{13}C NMR (DMSO-d_6, 75 MHz): δ 182.1 (s, C-4), 164.2 (s, C-2), 161.3 (s, C-4′), 156.3 (s, C-7), 152.3 (s, C-9), 152.0 (s, C-5), 132.5 (s, C-6), 128.4 (d, C-2′and C-6′), 121.0 (s, C-1′), 115.9 (d, C-3′ and C-5′), 105.6 (s, C-10), 102.6 (d, C-3), 100.2 (d, Glc-1), 94.3 (d, C-8), 77.2 (d, Glc-3), 76.7 (d, Glc-5), 73.1 (d, Glc-2), 69.6 (d, Glc-4), 60.6 (t, Glc-6), and 60.2 (q, OCH_3) are identical with the literature (Cuvelier et al., 1996; Agrawal, 1989).

*(−)-Isolariciresinol 3α-O-β-D-glucopyranoside (**6**).* Amorphous powder; $[\alpha]_D^{22}$ −31.1 °C (c 0.22, CD_3OD); DCI−MS *m/z*: 540 $[M + NH_4]^+$; ^{1}H NMR (300 MHz, CD_3OD): δ 6.74 (1H, d, J = 8.0 Hz, H-5′), 6.69 (1H, d, J = 2.0 Hz, H-2′), 6.65 (1H, s, H-8), 6.64 (1H, dd, J = 8.0; 2.0 Hz, H-6′), 6.19 (H, s, H-5), 4.04 (1H, d, J = 7.6 Hz, H-1″), 3.50−3.90 (m), 3.81 (3H, s, OCH_3), 3.78 (3H, OCH_3), 2.70−3.30 (m), 1.97 (2H, m, H-2, H-3). ^{13}C NMR (CD_3OD, 75 MHz): δ 148.9 (s, C-3′), 147.2 (s, C-7), 145.9 (s, C-6), 145.2 (s, C-4′), 138.7 (s, C-1′), 133.7 (s, C-10), 129.2 (s, C-9), 123.5 (d, C-6′), 117.3 (d, C-5), 115.9 (d, C-5′), 113.9 (d, C-2′). 112.3 (d, C-8), 103.8 (d, C-1″), 78.2 (d, C-5″), 77.8 (d, C-3″), 75.0 (d, C-2″), 71.4 (d, C-4″), 70.7 (t, C-3a), 65.5 (t, C-2a), 62.8 (t, H-6″), 56.4 (q, OCH_3), 56.5 (q, OCH_3), 48.3 (d, C-4), 45.3 (d, C-3), 41.1 (d, C-2), and 33.6 (t, C-1) are identical with the literature (Achenbach et al., 1992).

*Luteolin-7-O-β-glucopyranoside (**7**).* Yellow powder; FAB−MS *m/z*: $[M + 1]^+$, 449; ^{1}H NMR (DMSO, 200 MHz): δ 7.49 (1H, d, J = 8.0 Hz, H-6′), 7.44 (1H, s, H-2′), 6.92 (1H, d, J = 8.0, H-5′), 6.80 (1H, s, H-3), 6.76 (1H, br s, H-8), 6.46 (1H, br s, H-6), 5.10 (1H, d, J = 6.6 Hz, Glc-1), 3.10−3.80 (m). ^{13}C NMR (DMSO, 50 MHz): δ 182.2 (s, C-4), 164.8 (s, C-7), 163.2 (s, C-2), 161.4 (s, C-5), 157.2 (s, C-9), 150.2 (s, C-4′), 146.0 (s, C-3′), 121.6 (s, C-1′), 119.4 (d, C-6′), 116.2 (d, C-5′), 113.8 (d, C-2′), 105.6 (s, C-10), 103.4 (d, C-3), 100.1 (d, Glc-1), 99.8 (d, C-6), 95.0 (d, C-8), 77.4 (d, Glc-5), 76.7 (d, Glc-3), 73.4 (d, Glc-2), 69.8 (d, Glc-4), and 60.9 (t, Glc-6) are identical with the literature (Agrawal, 1989).

*4-Hydroxyacetophenone-4-O-β-D-apiofuranosyl-(1→6)-O-β-D-glucopyranoside (**8**).* Amorphous powder; FAB-MS *m/z*: 453 $[M + Na]^+$; FT−IR (KBr): 3411 (OH), 1669 (CO), 1601, 1510, 1250 cm^{-1}; ^{1}H NMR (CD_3OD, 200 MHz): δ 8.01 (2H, dd, J = 8.2; 1.6 Hz, H-4, H-8), 7.15 (2H, dd, J = 8.2, 1.6 Hz), 5.03 (1H, d, J = 7.6 Hz, H-1′), 4.98 (1H, d, J = 2.6 Hz, H-1″), 3.31−4.10 (11H, m), 2.57 (3H, s). ^{13}C NMR see Table 1.

*Icariside F2 (**9**).* Amorphous powder; FAB−MS *m/z*: 425 $[M + Na]^+$; ^{1}H NMR (CD_3OD, 200 MHz): δ 7.30−7.70 (5H, m, H-2; 3; 4; 5; 6), 5.06 (1H, d, J = 2.6 Hz, H-1″), 4.93 (1H, d, J = 12.0 Hz, H-7), 4,66 (1H, d, J = 12.0 Hz, H-7). 4.35 (1H, d, J = 7.4 Hz, H-1′), 3.10−4.10 (m, 11H). ^{13}C NMR, see Table 1, are identical with the literature (Miyase et al., 1988).

*2,3-Dihydro-2-(4′-hydroxy-3′-methoxyphenyl)-3-(hydroxymethyl)-7-methoxy-5-benzofuranpropanol 4′-O-β-glucopyranoside (**10**).* $[\alpha]_D^{22}$ −33.4 °C (c 0.25, CD_3OD); amorphous powder; FAB−MS *m/z*: 545 $[M + Na]^+$; ^{1}H NMR (CD_3OD, 200 MHz): 7.14 (1H, d, J = 8.4 Hz, H-5′), 7.04 (1H, d, J = 2.0, H-2′), 6.91 (1H, dd, J = 8.4; 2.0 Hz, H-6′), 6.72 (2H, s, H-2 and H-6), 5.56 (1H, d, J = 5.8 Hz, H-7′), 3.83 (3H, s, OCH_3), 3.81 (3H, OCH_3), 3.2−3.9 (11H, m), 2.63 (2H, m H-7), 1.78 (2H, H-8). ^{13}C NMR (CD_3OD, 50 MHz): 151.2 (s, C-4′), 147.9 (s, C-3′), 147.7 (s, C-4), 145.5 (s, C-3), 138.6 (s, C-1′), 137.4 (s, C-5), 129.9 (s, C-1), 119.7

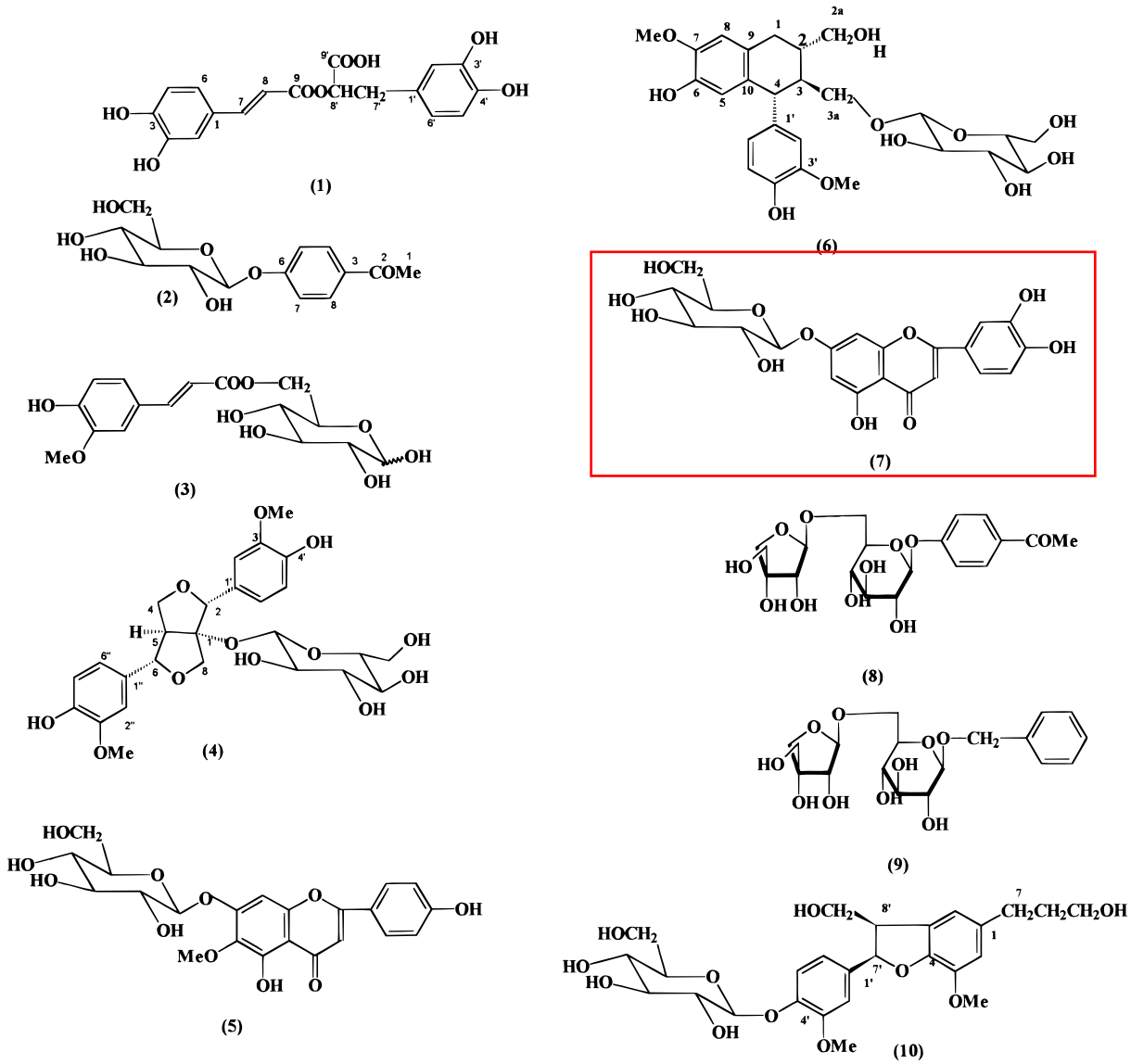

Figure 1. Structures of 10 compounds identified in sage.

(d, C-6′), 118.2 (d, C-2), 118.2 (d, C-5′), 114.4 (d, C-6), 111.4 (d, C-2′), 103.0 (d, Glc-1), 88.8 (d, C-7′), 78.5 (d, Glc-3), 78.1 (d, Glc-5), 75.1(d, Glc-2), 71.6 (d, Glc-4), 65.3 (t, C-9′), 62.8 (t, Glc-6), 62.6 (t, C-9), 2 × 57.0 (q, OCH_3), 55.9 (d, C-8′), 36.1 (t, C-8), and 33.2 (t, C-7) are identical with the literature (Lundgren et al., 1981). Its ^{13}C NMR data was assigned according to literature (Wang and Jia, 1997).

Determination of the Scavenging Effect on DPPH Radicals. This method was adapted from Chen et al. (1997). In the 1.0 × 10^{-4} M ethanol solution of DPPH, tested compounds were added, and their final concentrations were 20 μM. Then the solution with tested samples was shaken vigorously and kept in the dark for 0.5 h. The absorbance of the samples was measured on a spectrophotometer (Milton Roy, model 301) at 517 nm against a blank of ethanol without DPPH. All tests were run in triplicate and averaged.

Measurement of Radical Cation $ABTS^{\bullet+}$ Scavenging Activity. The radical cation $ABTS^{\bullet+}$ scavenging activity was measured using commercial kits from Randox Laboratories Ltd. (San Francisco, CA). Phosphate-buffered saline (80 mM) was used as a buffer. Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) (1.6 mM) was prepared in DMSO for use as an antioxidant standard. The 1 mM sample solutions were prepared in DMSO. The concentrations in this test were 3.1 μM for metmyoglobin, 305 μM for 2,2′-azinobis(3-ethylbenzothiozoline-6-sulfonate) (ABTS), and 125 μM for H_2O_2. The sample or Trolox (20 μL) was mixed with chromogen (1 mL of metmyoglobin and ABTS mixture) in a cuvette, and a spectrophotometer (Milton Roy, model 301) on 600 nm was used to get initial absorbance A1, and then 200 μL of H_2O_2 was added to this cuvette and held at 40 °C for exactly 15 min before the absorbance A2 was read. The following equation was used to calculate the Trolox equivalent:

$$(\text{mM}) = \text{Trolox concentration (mM)} \times (\Delta A_{\text{blank}} - \Delta A_{\text{sample}})/(\Delta A_{\text{blank}} - \Delta A_{\text{trolox}})$$

where ΔA = A2−A1.

RESULTS AND DISCUSSION

Repeated silica gel, RP-18, RP-8, and Sephadex LH-20 column chromatography of a water-saturated butanol fraction of sage extract led to the isolation of 10 phenolic compounds. Their structures were determined by spectral methods to be rosmarinic acid (**1**), 4-*O*-*β*-D-glucopyranosylacetophenone (picein, **2**), 6-*O*-(*E*)-feruloyl-(α and *β*)-glucopyranoside (**3**), (+)-1-hydroxypinoresinol-1-*β*-D-glucoside (**4**), homoplantaginin.(**5**), (−)-isolariciresinol 3α-*O*-*β*-D-glucopyranoside (**6**), luteolin-7-*O*-*β*-glucopyranoside (**7**), 4-hydroxyacetophenone-4-*O*-*β*-D-apiofuranosyl-(1→6)-*O*-*β*-D-glucopyranoside (**8**), icariside F2 (**9**),

Table 2. Scavenging Effects of Antioxidants on the DPPH Free Radical and Radical Cation ABTS$^{\bullet+}$

tested compd (1.0 mM)	Trolox equivalent (mM) (SD)[b]	DPPH inhibition percentage (SD)[a]
rosmarinic acid (**1**)	5.07 (0.01)	88.2 (1.2)
4-*O*-*β*-D-glucopyranosylacetophenone (picein, **2**)	0.72 (0.02)	*c*
6-*O*-(*E*)-feruloyl-(α and *β*)-glucopyranoside (**3**)	2.74 (0.08)	11.0(1.8)
(+)-1-hydroxypinoresinol-1-*β*-D-glucoside (**4**)	3.20 (0.09)	*c*
homoplantaginin (**5**)	3.83 (0.06)	*c*
(−)-isolariciresinol 3α-*O*-*β*-D-glucopyranoside (**6**)	3.38 (0.03)	21.8 (1.7)
luteolin-7-*O*-*β*-glucopyranoside (**7**)	4.80 (0.05)	62.0 (1.1)
4-hydroxyacetophenone-4-*O*-*β*-D-apiofuranosyl-(1→6)-*O*-*β*-D-glucopyranoside (**8**)	0.53 (0.02)	*c*
icariside F2 (**9**)	0.64 (0.05)	*c*
2,3-dihydro-2-(4′-hydroxy-3′-methoxyphenyl)-3-(hydroxymethyl)-7-methoxy-5-benzofuranpropanol 4′-*O*-*β*-glucopyranoside (**10**)	0.50 (0.10)	*c*

[a] Scavenging effects of the antioxidant on DPPH radicals, the concentration of DPPH ethanolic solution was 1.0×10^{-4} M. The concentration of antioxidants are 20 μM; each value is the mean of triplicate measurements. [b] Scavenging effects on and radical cation ABTS$^{\bullet+}$ are expressed as Trolox equivalent (mM). [c] No activity observed at 20 μM.

and 2,3-dihydro-2-(4′-hydroxy-3′-methoxyphenyl)-3-(hydroxymethyl)-7-methoxy-5-benzofuranpropanol 4′-*O*-*β*-glucopyranoside (**10**). The structures of the 10 compounds identified are shown in Figure 1.

Compound **8** was isolated as amorphous powder, and the molecular formula $C_{19}H_{26}O_{11}$ was deduced from FAB−MS and ^{13}C NMR spectra. Comparing the ^{13}C NMR and ^{1}H NMR spectra with those of 4-*O*-*β*-D-glucopyranosylacetophenone (picein, **2**) suggested the presence of acetophenone moiety in compound **8**. Like icariside F2 (**9**), the ^{13}C NMR and ^{1}H NMR spectra of compound **8** showed the existence of *β*-glucosyl and *β*-apiosyl moieties, and the two sugars were connected as *β*-D-apiofuranosyl-(1→6)-*O*-*β*-D-glucopyranose. Compound **8** was, therefore, elucidated as 4-hydroxyacetophenone-4-*O*-*β*-D-apiofuranosyl-(1→6)-*O*-*β*-D-glucopyranoside.

Scavenging DPPH Free Radicals. Usually screening antioxidative activity in various model systems is important prior to the application of antioxidants in food. Free radical scavenging is one of generally accepted mechanisms against lipid oxidation. The effect of antioxidants on DPPH radical scavenging was thought to be due to their hydrogen donating ability (Baumann et al., 1979). As shown in Table 2, four compounds (**1**, **3**, **6**, and **7**) showed radical scavenging activity. The scavenging activity order of the test compounds was **1** > **7** > **6** > **3**. It has been reported that the antioxidative activity of polyphenols is related to their hydroxyl group and the presence of a second hydroxyl group in the *ortho* or *para* position is known to increase the antioxidative activity due to additional resonance stability and *o*-quinone or *p*-quinone formation (Chen and Ho, 1997; Bouchet et al., 1998). This explains why compounds **1** and **7** are active free radical scavenging agents.

Measurement of Radical Cation ABTS$^{\bullet+}$ Scavenging Activity. This method measures the relative ability of antioxidant substances to scavenge the radical cation of 2,2′-azinobis(3-ethylbenzothiozoline-6-sulfonate) (ABTS$^{\cdot+}$) in the aqueous phase as compared to a standard amount of the synthetic antioxidant Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), the water-soluble vitamin E analogue (Miller et al., 1993, 1995; Rice-Evans et al., 1995, 1996; Salah et al., 1995). In this method, the activity of tested compounds was expressed as a Trolox equivalent—the millimolar concentration of a Trolox solution having an antioxidant capacity equivalent to a 1.0 mM solution of the substance under investigation. As shown in Table 2, all these compounds have antioxidative activity as compared with Trolox. As in the DPPH model, rosmarinic acid (**1**) and luteolin-7-*O*-*β*-glucopyranoside (**7**) are the most active antioxidants and 4-*O*-*β*-D-glucopyranosylacetophenone (picein, **2**), 4-hydroxyacetophenone-4-*O*-*β*-D-apiofuranosyl-(1→6)-*O*-*β*-D-glucopyranoside (**8**), icariside F2 (**9**), and 2,3-dihydro-2-(4′-hydroxy-3′-methoxyphenyl)-3-(hydroxymethyl)-7-methoxy-5-benzofuranpropapol 4′-*O*-*β*-glucopyranoside (**10**) showed less activity. Only (+)-1-hydroxypinoresinol-1-*β*-D-glucoside (**4**) and homoplantaginin (**5**) showed different antioxidant activities in these two models.

Phenolic compounds are widely distributed in nature. It is suggested that their antioxidative activity is related to their conjugated rings and hydroxyl groups (Decker, 1995), so it is not a surprise that most of phenolic compounds from sage showed some antioxidative activity. Among these compounds, rosmarinic acid and luteolin-7-O-*β*-glucopyranoside are the most active. The antioxidant activity of rosmarinic acid has been studied extensively (Chen and Ho, 1997; Pearson, 1997; Kikuzaki and Nakatani, 1989). To our knowledge, the antioxidative activity of the other compounds identified have not been reported.

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Received for review June 12, 1998. Revised manuscript received October 6, 1998. Accepted October 13, 1998.

JF980614B